```
     (FILE 'USPAT' ENTERED AT 16:08:30 ON 22 MAR 1999)
L1           929 S (NUCLEATE? OR NUCLEUS)(P)(BLOOD)
L2             1 S L1(P)QBC
L3            67 S L1(P)(CANCER OR EPITHELIAL OR NEOPLAS?)
L4            79 S L1(P)(DENSITY OR GRAVITAT? OR GRAVIMETRIC? OR SPECIFIC(W
)GR
L5            32 S L4(P)CENTRIFUG?
L6          5919 S (CAPILLARY OR TUBE)(P)FLOAT
L7           191 S L6(P)(SPECIFIC(W)GRAVITY)
L8            22 S L7(P)(ANNUL?)
                 E AU=RIMM DAVID L?
```

Set	Items	Description
S1	9563	(NUCLEATE? OR NUCLEUS)(5N)(BLOOD)
S2	0	S1(5N)QBC
S3	110	S1(5N)(CANCER OR EPITHELIAL OR NEOPLAS?)
S4	71	RD (unique items)
S5	56	S1(5N)(DENSITY OR GRAVITAT? OR GRAIMETRIC? OR SPECIFIC(W)GRAVITY)
S6	41	RD (unique items)
S7	0	S1(5N)(TUBE(5N)(ZONE OR ANNULAR))
S8	6724	((EPITHELIAL OR CANCER)(5N)(CELLS))(5N)BLOOD
S9	14	S8(5N)(DENISTY OR GRAVITAT? OR GRAVIMETRIC? OR SPECIFIC(W)DENSITY OR CENTRIFUG?)
S10	7	RD (unique items)
S11	2015	(CAPILLARY OR TUBE)(5N)(FLOAT)
S12	11	S11(5N)(SPECIFIC(W)GRAVITY OR ANNULAR(W)FREE(W)ZONE)
S13	11	RD (unique items)
S14	258	E3,E5,E6,E8,E9,E10,E12
S15	13	S14 AND (NUCLEAT? OR NUCLEUS)

01305929 SUPPLIER NUMBER: 09863873 (THIS IS THE FULL TEXT)
Rapid diagnosis of malaria by fluorescence microscopy with light microscope and interference filter.

Kawamoto, Fumihiko
The Lancet, v337, n8735, p200(3)
Jan 26,
1991

TEXT:

Introduction

The establishment of new diagnostic methods is a major goal in the malaria eradication programme. Immunological (immunofluorescence antibody [IFA]/enzyme-linked-immunosorbent assay [ELISA] methods, [1,2] DNA or RNA probes, [2,3] and fluorochrome staining techniques [4-7] have been developed in many laboratories, 4',6-diamidino-2-phenylindole/propidium iodide (DAPI/PI) [4,5] and acridine orange (AO) staining in the '**QBC**' tube system [6,7] are reportedly more sensitive than Giemsa staining for detection of malaria parasites. In both staining methods, differential staining of nuclear DNA and cytoplasmic RNA leads to reliable and easy identification of the parasites. In particular, the outlines of stained parasites are well preserved and the general **morphology** is similar to that in specimens stained by Giemsa, [5] though pigment granules are not stained. Furthermore, fluorochrome staining techniques are much easier for inexperienced workers and are less time-consuming. The major drawback of fluorochrome staining methods is thta an expensive fluorescence microscope is needed. In the '**QBC**' method, a cheaper fluorescence microscope system (Becton Dickinson, USA), consisting of a battery-powdered light and a special ultraviolet lens, has been developed with a battery-powered centrifuge, but this system is still prohibitively expensive for tropical countries where malaria is endemic.

Fluorescence microscopy with an interference filter system (a multi-layered excitation filter combined with a barrier filter) was developed initially to look at fluorescein isothiocyanate (FITC)-labelled immunofluorescence in the standard light microscope. [8,9] This system can be applied to fluorochromes, the excitation wavelengths of which are over 400 nm (B-excitation and G-excitation systems), and can easily detect the emitted fluorescence. Thus, if (as in the FITC interference system) a well designed interference filter were fitted for a certain fluorochrome, the fluorescence filter were fitted for a certain fluorochrome, the fluorescence might be visible with a standard light microscope. Here, I describe a new interference filter, designed for use with AO in a standard light microscope, and how it could be applied to rapid diagnosis of malarias.

Materials and methods

AO fluoresces green (535 nm) and red (650 nm) when excited at 430 nm and 492-495 nm, respectively. [5,10] A new interference filter (patent submitted) was made for these excitation wavelengths, and a barrier filter with a cut-off wavelength of 515 nm was inserted into the eye pieces of light microscopes.

Thick and thin blood smears from patients with malaria were obtained from several laboratories in Japan and the UK. The slides had been kept for 1-5 years without staining after fixation with methanol. Staining was done by addition of a drop of AO solution (10-50 [milligram]/ml in 0.05 mol/l "tris"-hydrochloric acid buffer, pH 7.4) or by immersion of slides into this solution for 1 s; a cover slip was put onto the preparation with the

stain solution as a mo[redacted]ing medium. For comparison, so[redacted]smears were also stained with Giemsa. O[redacted]rvations were made with light m[redacted]roscopes (Olympus BH-2, Nikon optiphot, and Leiz FS 2929, 50-100 W halogen-lights), which had standard lenses (x 10 ocular lensa dn x 20-40 objectives without immersion oil).

Results

The figure shows fluorescence micrographs of Plasmodium falciparum and P vivax in thick and thin blooodfilms taken by the interference filter system. As expected, nuclei of malaria parasites and leucocytes fluoresced green, and the cytoplasm of the parasites and lymphocytes were bright red--stronger than that of other leucocytes--in the dark background. Malaria parasites could easily be distinguished from leucocytes because of the pronounced differences in size, shape, and staining properties. All the developmental stages of malaria parasites were easily identified at a magnification of x400. Additionally, because of the bright red fluorescence of the cytoplasm, which was especially intense in young and matured gametocytes, scanning of thin smears at a magnification of x200 could be done rapidly. Even at low parasitaemia (< 0.0002%; 1 to several parasites/thin smear), parasites could be detected within about 15 min if the whole of each thin smear was scanned (x 200). Time required to find the first parasite in these smears ranged from 20 s to 14 min (n=5, data not shown), which was much faster than that by Giemsa at x1000. In some Giemsa-stained smears, the whole cells were stained strongly blue probably because of long and poor storage, and it was difficult to detect the parasites in these smears. In such cases, however, there was no difficulty in detecting parasites by the filter system with AO. Concentrations of AO solution higher than 100 [milligram]/ml were inadequate since the nuclei of the parasites were frequently stained red or orange. [5]

Fluorescence intensity emitted by the interference filter system was weaker than that of an epi-illuminated, mercury vapour, fluorescence microscope (BH-2 with B-excitation system), but the **morphology** of the parasites was the same, and easily detected by both fluorescence microscopy methods.

Discussion

The interference filter system designed for use with AO in a light microscope is useful, economic, and easy to use for the laboratory diagnosis of malarias. Inexperienced microscopists may have difficulty reading thin Giemsa-stained smears and often cannot read thick smears. However, with AO staining, reading of both thin and thick smears is easier since artifacts or pigment dots, which are frequently troublesome in the Giemsa stain, cannot be seen. Furthermore, since AO mostly did not react with erythrocytes and platelets, the **morphology** of fluorescing malaria parasites in the dark field was easily identified at lower magnifications. There is a great advantage to this staining because, as in the DAPI/PI method, [4,5] the **morphology** seen by differential staining of the nuclei and the cytoplasm is similar to that seen with Giemsa staining, and therefore, like the **QBC** technique, this new method is easier for beginners to learn. For example, untrained medical students could easily detect malaria parasites in blood smears after a 5-20 min demonstration. Additionally, rapid detection of Trypanosoma brucei and microfilariae of Brugia malayi and B pahangi from thin bloodfilms was also much easier than by Giemsa staining (unpublished).

The AO staining solution is stable for years when kept in the dark even in tropical areas. Additionally, AO-stained specimens can be re-stained many times either with AO or with Giemsa [5] after rinsing in methanol. A new "thick smear" method, in which blood samples are simultaneously haemolysed and stained with AO (unpublished) has also been developed.

The reason why fluorescences obtained by the interference filter and halogen lights were weaker than those obtained with standard fluorescence microscopy and a mercury lamp is that the intensity of fluorescence is proportional to the excitation energy. [9,10] However, fluorescences emitted by the interference system were strong enough to detect all the parasites tested, and the system is much cheaper than standard fluorescence microscopes. Additionally, I have been able to detect parasites more

clearly using the interference filter in a daylight illuminated light microscope than a halogen illuminated microscope (unpublished). The interference filter could possibly be used as an FITC filter for immunological investigations since it transmits an excitation wavelength of FITC (490nm). By contrast, a commercially available FITC filter can be used for this system only if its red diffraction beam of 645-700 nm (known as "red light for contrasting dark-field") is suppressed by an additional filter. [9,10] Use of three suppression filters with the FITC filter (Olympus) is essential to obtain results similar to those for the AO filter (unpublished); moreover, these four filters could not be used in daylight illuminated microscopes. Improvement of the filter system to obtain a more intense fluorescence is now in progress.

I thank Prof A. Ishii, Okayama University, Japan, for his valuable suggestion, and Dr P. F. Billingsley, Imperial College, London, for his critical appraisal of the manuscript. This study received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

REFERENCES

[1] Voller A, Draper CC. Immunodiagnosis and seroepidemiology of malaria. Br Med Bull 1982; 38:173-77.

[2] Bruce-Chwatt LJ. From Laveran's discovery to DNA probes: new trends in diagnosis of malaria. Lancet 1987; ii:1509-11.

[3] Barker RH, Suebsang L, Roney W. Specific DNA probe for the diagnosis of P falciparum malaria. Science 1986; 231:1434-36.

[4] Kawamoto F, Mizuno S, Fujioka H, et al. Simple and rapid staining for detection of Entamoeba cysts and other protozoans with fluorochromes. Jpn J Med Sci Biol 1987; 40:35-46.

[5] Kawamoto F, Kumada N. Fluorescent probes for detection of protozoan parasites. Parasitol Today 1987; 3:284-86.

[6] Spielman A, Prerrone JB, Teklehaimanot A, et al. Malaria diagnosis by direct observation of centrifuged samples of blood. Am J Trop Med Hyg 1988; 39:337-42.

[7] Rickman LS, Long GW, Oberst R, et al. Rapid diagnosis of malaria by acridine orange staining of centrifuged parasites. Lancet 1989; i: 68-71.

[8] Rygaard J, Olsen W. Interference filters for improved immunofluorescence microscopy. Acta Path Microbiol Scand 1969; 76:146-49.

[9] Fluorescence microscopy. In: Kawamura A Jr, ed. Fluorescent antibody techniques and their applications. Tokyo: University of Tokyo Press, 1977; 95-114.

[10] Rost FW. Fluorescence microscopy. In: Everson Pearse AG, ed. Histochemistry theoretical and applied. Vol 1. Preparative and optical technology. Edinburgh: Churchill Livingstone, 1980; 346-78.

CAPTIONS: Fluorescence micrographs of malaria parasites. (diagnostic image)

6/7/3 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07059627 BIOSIS NO.: 000089129731
RAPID DIAGNOSIS OF BRUGIA-MALAYI AND WUCHERERIA-BANCROFTI FILARIASIS BY AN ACRIDINE ORANGE-MICROHEMATOCRIT TUBE TECHNIQUE

AUTHOR: LONG G W; RICKMAN L S; CROSS J H
AUTHOR ADDRESS: INFECTIOUS DISEASES DEP., NAVAL MED. RES. INST., BETHESDA, MD. 20814-5055.

JOURNAL: J PARASITOL 76 (2). 1990. 278-281.
FULL JOURNAL NAME: Journal of Parasitology
CODEN: JOPAA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A microhematocrit tube technique for diagnosis of human filariasis has been previously described. A system incorporating heparin, EDTA, and acridine orange into a microhematocrit tube (Quantitative Blood Count, **QBC**) has been commercially developed for the quantitation of blood counts and has been used for the diagnosis of malaria. We evaluated this test for its usefulness in the diagnosis of filariasis. Upon centrifugation, the parasites were concentrated in the area of the buffy coat and could be observed through the wall of the tube. The parasites were concentrated further by a plastic float that expands the buffy coat and confines the parasites to the periphery of the tube. Acridine orange stains the DNA of the parasite, and **morphologic** characteristics can be examined by fluorescence microscopy. The terminal and subterminal nuclei and long cephalic space of Brugia malayi, as well as the short cephalic space and caudal nuclei of Wuchereria bancrofti, were easily recognized and differentiated from each other. Microfilariae were

SYSTEM:OS - DIALOG OneSearch
File 149:IAC(SM)Health&Wellness DB(SM) 1976-1999/Mar W3
(c) 1999 Info Access Co
File 5:BIOSIS PREVIEWS(R) 1969-1999/Mar W3
(c) 1999 BIOSIS
File 73:EMBASE 1974-1999/Feb W2
(c) 1999 Elsevier Science B.V.
*File 73: EMBASE reloaded. Accession numbers have changed.
EXPLODE now retrieves ALL narrower terms. See HELP NEWS73.
File 156:Toxline(R) 1965-1998/Dec
(c) format only 1999 The Dialog Corporation
File 159:Cancerlit 1975-1999/Feb
(c) format only 1999 Dialog Corporation
File 155:MEDLINE(R) 1966-1999/May W2
(c) format only 1999 Dialog Corporation
*File 155: reloaded, note accession numbers changed.
File 434:SciSearch(R) Cited Ref Sci 1974-1989/Dec
(c) 1998 Inst for Sci Info
File 76:Life Sciences Collection 1982-1999/Jan
(c) 1999 Cambridge Sci Abs
File 652:US Patents Fulltext 1971-1979
(c) format only 1999 The Dialog Corp.
*File 652: Reassignment data now current through 08/20/98
Reexamination, extension, expiration, reinstatement updated weekly.
File 653:US Patents Fulltext 1980-1989
(c) format only 1999 The Dialog Corp.
*File 653: Reassignment data now current through 08/20/98.
Reexamination, extension, expiration, reinstatement updated weekly.
File 654:US Pat.Full. 1990-1999/Mar 16
(c) format only 1999 The Dialog Corp.
*File 654: Reassignment data now current through 08/20/98.
Reexamination, extension, expiration, reinstatement updated weekly.
File 6:NTIS 64-1999/Apr W3
Comp&distr 1998 NTIS, Intl Copyright All Righ
File 34:SciSearch(R) Cited Ref Sci 1990-1999/Mar W2
(c) 1999 Inst for Sci Info
File 40:Enviroline(R) 1975-1998/Oct
(c) 1998 Congressional Information Service
File 41:Pollution Abs 1970-1999/Apr
(c) 1999 Cambridge Scientific Abstracts
File 50:CAB Abstracts 1972-1999/Feb
(c) 1999 CAB International
File 65:Inside Conferences 1993-1999/Mar W2
(c) 1999 BLDSC all rts. reserv.
File 68:Env.Bib. 1974-1999/Mar
(c) 1999 Internl Academy at Santa Barbara
File 71:ELSEVIER BIOBASE 1994-1999/Feb W1
(c) 1999 Elsevier Science B.V.
File 77:Conference Papers Index 1973-1999/Mar
(c) 1999 Cambridge Sci Abs
File 94:JICST-EPlus 1985-1999/Dec W4
(c)1999 Japan Science and Tech Corp(JST)
File 98:General Sci Abs/Full-Text 1984-1999/Feb
(c) 1999 The HW Wilson Co.
File 103:Energy SciTec 1974-1999/Oct B2
(c) 1999 Contains copyrighted material
*File 103: Energy Science and Technology updates have been delayed.
We are actively working with the producer to solve this issue.

File 143:Biol. & Agr[redacted]. Index 1983-1999/Feb
(c) 1999 The[redacted] Wilson Co
File 144:Pascal 1973-1999/Jan
(c) 1999 INIST/CNRS
File 162:CAB HEALTH 1983-1999/Jan
(c) 1999 CAB INTERNATIONAL
File 172:EMBASE Alert 1999/Mar W2
(c) 1999 Elsevier Science B.V.
File 305:Analytical Abstracts 1980-1999/Apr
(c) 1999 Royal Soc Chemistry
File 370:Science 1996-1999/Feb W1
(c) 1999 AAAS
File 399:CA SEARCH(R) 1967-1999/UD=13012
(c) 1999 American Chemical Society
*File 399: Use is subject to the terms of your user/customer agreement.
RANK charge added; see HELP RATES 399.
File 8:Ei Compendex(R) 1970-1999/Mar W1
(c) 1999 Engineering Info. Inc.
File 60:CRIS/USDA 1998/Sep
(c) format only 1998 The Dialog Corporaion pl
*File 60: This file will no longer be updated.
File 99:Wilson Appl. Sci & Tech Abs 1983-1999/Feb
(c) 1999 The HW Wilson Co.
File 266:FEDRIP 1999/Jan
Comp & dist by NTIS, Intl Copyright All Rights Res
File 315:ChemEng & Biotec Abs 1970-1999/Mar
(c)1999 RoySocChm,DECHEMA,FizChemie
File 357:Derwent Biotechnology Abs 1982-1999/Mar B2
(c) 1999 Derwent Publ Ltd
*File 357: Effective October 1, DialUnit rates adjusted for unrounding.
See HELP NEWS 357 for details.
File 358:Current BioTech Abs 1983-1999/Apr
Royal Soc Chem & DECHEMA

/7/11 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE

06005088 EMBASE No: 1995033740
Model study detecting breast cancer cells in peripheral blood mononuclear cells at frequencies as low as 10sup -sup 7
Gross H.-J.; Verwer B.; Houck D.; Hoffman R.A.; Recktenwald D.
Monoclonal Research Center, B. Dickinson Immunocytometry Systems, 2350 Qume Drive,San Jose, CA 95131-1807 United States
Proceedings of the National Academy of Sciences of the United States of America (PROC. NATL. ACAD. SCI. U. S. A.) (United States) 1995, 92/2 (537-541)

CODEN: PNASA ISSN: 0027-8424
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

A flow cytometric assay was developed to detect rare cancer cells in blood and bone marrow. Multiple markers, each identified by a separate color of immunofluorescence (yellow and two shades of red), are used to reliably identify the cancer cells. Blood or bone marrow cells, which are not of interest but interfere in detecting the cancer cells, are identified by a panel of immunofluorescence markers, each of which has the same color (green). Thus, the rare cancer cells of interest are yellow and two different shades of red but not green. The requirement that the rare cancer cell be simultaneously positive for three separate colors (the specific markers) and negative for a fourth color (the exclusion color) allowed detection of as few as one **cancer** cell in 10sup 7 **nucleated blood** cells (a frequency of 10sup -sup 7). To test this rare-event assay prior to clinical studies, a model study was performed in which the clinical sample was simulated by mixing small numbers of cells from the breast carcinoma line BT-20 with peripheral blood mononuclear cells. We detected statistically significant numbers of BT-20 cells at mixing frequencies of 10sup -sup 5, 10sup -sup 6, and 10sup -sup 7. In control samples, no target events (BT-20) were observed when more than 10sup 8 cells were analyzed. For additional confirmation that the BT-20 cells in the model study were correctly identified and counted, the BT-20 cells (and only BT-20 cells) were covalently stained with a fifth fluorescent dye, 7-amino-4- chloromethylcoumarin (CMAC). CMAC fluorescence data were not used in the assay for detecting BT-20 cells. Only after the analysis using data from the specific and exclusion colors had been completed were the events identified as BT-20 cells checked for CMAC fluorescence. The putative BT-20 events were always found to be positive for CMAC fluorescence, which further increases confidence in the assay. Manual data analysis and an automated computer program were compared. Results were comparable with the manual and automated methods, but the automated 'genetic algorithm' always found more BT-20 events. Cell sorting of BT-20 cells from samples that contained BT-20 at frequencies of 10sup -sup 5, 10sup -sup 6, and 10sup -sup 7 provided further evidence that these rare cells could be reliably detected. The good performance of the assay with

10/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10872512 BIOSIS NO.: 199799493657
Experimental isolation of prostate **epithelial cells** from total **blood** using **gravitational** and sedimentation field-flow fractionation.

AUTHOR: Dumas Frederic(a); Le Maire Valerie; Eschwege Pascal; Benoit Gerard ; Jardin Alain; Lacour Bernard; Cardot Philippe; Loric Sylvain
AUTHOR ADDRESS: (a)Paris, France

JOURNAL: Journal of Urology 157 (4 SUPPL.):p344 1997

CONFERENCE/MEETING: 92nd Annual Meeting of the American Urological Association New Orleans, Louisiana, USA April 12-17, 1997
ISSN: 0022-5347
RECORD TYPE: Citation
LANGUAGE: English

10/7/3 (Item 1 from file: 159)
DIALOG(R)File 159:Cancerlit

00814871 91360039 MEDL/91360039
[AUTOTRANSFUSION IN HEPATECTOMY FOR HEPATOCELLULAR CARCINOMA]
Fujimoto J; Okamoto E; Yamanaka N; Oriyama T; Furukawa K; Kawamura E; Tanaka T
First Department of Surgery, Hyogo College of Medicine, Nishinomiya, Japan.
Nippon Geka Gakkai Zasshi; 92(7):825-30 1991 ISSN 0301-4894
Journal Code: NGG
Languages: JAPANESE
Document Type: JOURNAL ARTICLE English Abstract
Experimental data of autotransfusion in hepatectomy for hepatocellular carcinoma and clinical data from forty-eight patients were analyzed. By a density gradient medium, cancer cells banded at a lower density (1.056 g/ml: mean) than red blood cells (1.105 g/ml: mean). The **centrifuge** autotransfusor separated the red **blood cells** from **cancer cells** and all sections of cell blocks of the blood harvested intraoperatively in 20 cases revealed no malignant cells. The average requirement of bank blood was significantly reduced in these patients than in patients without autotransfusion during one year before, 1573 ml to 478 ml (whole blood of concentrated red blood cells) (p less than 0.05). These findings indicate that autotransfusion in hepatectomy for hepatocellular carcinoma is safe and practical.

6/7/2 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10924695 BIOSIS NO.: 199799545840
Noninvasive prenatal diagnosis: Use of density gradient centrifugation, magnetically activated cell sorting and in situ hybridization.

AUTHOR: Campagnoli Cesare; Multhaupt Hinke A B; Ludomirski Abraham; Haut Michael J; Warhol Michael J(a)
AUTHOR ADDRESS: (a)Dep. Pathol., Pennsylvania Hosp., 800 Spruce St., Philadelphia, PA 19107, USA

JOURNAL: Journal of Reproductive Medicine 42 (4):p193-199 1997
ISSN: 0024-7758
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: OBJECTIVE: To develop a noninvasive method suitable for clinical prenatal diagnosis. STUDY DESIGN: Fetal nucleated erythrocytes were separated from peripheral blood of 17 healthy pregnant women using small magnetically activated cell sorting columns (MiniMACS) following density gradient centrifugation and dual antibody labeling methods. The protocol was designed to compare the efficacy of antitransferrin receptor (CD71)/antiglycophorin A (GPA) antibodies with antithrombospondin receptor (CD36)/anti-GPA antibodies in identifying nucleated erythrocytes in maternal blood. Cytospin preparations of the isolated cells were subjected to in situ hybridization with specific DNA probes for the Y chromosome and chromosome 21 to confirm the fetal origin. RESULTS: After MiniMACS the enrichment factor for the CD71/GPA- and CD36/GPA-positive cells from maternal blood were similar, and the percentages of fetal cells recovered did not differ. Seven of seven male pregnancies were correctly identified. One case of trisomy 21 was detected. CONCLUSION: The in situ hybridization analysis of fetal **nucleated** erythrocytes isolated from maternal **blood** using single **density** gradient centrifugation, antiCD71/anti-GPA immunostaining and MiniMACS could be an accurate, sensitive and noninvasive method for prenatal diagnosis.

6/7/4 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

06200641 BIOSIS NO.: 000086034823
A NEW MULTICHAMBER COUNTERFLOW CENTRIFUGATION ROTOR WITH HIGH-SEPARATION CAPACITY AND VERSATILE POTENTIALS

AUTHOR: PLAS A; DE WITTER T; WESSELS H; HAANEN C
AUTHOR ADDRESS: DIV. HAEMATOLOGY, DEP. INTERNAL MED., UNIV. HOSP., GEERT GROOTEPLEIN Z8, 6525 GA NIJMEGEN, THE NETHERLANDS.

JOURNAL: EXP HEMATOL (N Y) 16 (5). 1988. 355-359.
FULL JOURNAL NAME: Experimental Hematology (New York)
CODEN: EXHMA
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A new closed system for counterflow centrifugation (elutriation) is described. The system was developed to increase the capacity of counterflow centrifugation in order to be able to separate bone marrow intended for allogeneic bone marrow transplantation within 3 h. The rotor has the capacity for up to four separation chambers, offering the possibility of separating either a single-cell suspension under equal or differing conditions, or four different suspensions simultaneously. Profiles of low-**density nucleated** cells from normal **blood** were shown to be identical after elutriation in four different chambers. Leucocytes could be depleted from platelet concentrates without significant loss. Most (98%) of the lymphocytes were removed from donor marrow intended for transplantation within 3 h and the recovery of myeloid and erythroid clonogenic cells in the graft was similar to that obtained from the standard single chamber centrifuge.

6/7/10 (Item 4 from file: 73)
DIALOG(R)File 73:EMBASE

07081897 EMBASE No: 1997363760
Prenatal diagnosis of fetal trisomies 21 and triploidy using fetal cells in the maternal circulation
DIAGNOSI PRENATALE DI TRIPLOIDIA E TRISOMIA 21 MEDIANTE ARRICCHIMENTO DI ERITROBLASTI FETALI DAL SANGUE MATERNO
Pezzolo A.; Santi F.; Pistoia V.; De Biasio P.
A. Pezzolo, Laboratorio di Oncologia, Istituto Giannina Gaslini, Largo Gerolamo Gaslini, 5, 16148 Genova Italy
Minerva Medica (MINERVA MED.) (Italy) 1997, 88/10 (393-399)

CODEN: MIMEA ISSN: 0026-4806
DOCUMENT TYPE: Journal; Article
LANGUAGE: ITALIAN SUMMARY LANGUAGE: ENGLISH; ITALIAN
NUMBER OF REFERENCES: 23

Background. A long-sought goal of medical genetics has been the development of prenatal diagnostic procedures that do not endanger the conceptus. The safety of noninvasive methods for prenatal diagnosis would be especially attractive because they could be extended to all pregnant women, regardless of their ages or histories. Noninvasive prenatal diagnosis for the entire population might be possible recovering fetal cells from maternal blood. For this purpose, we have studied fetal erythroblasts. Materials and methods. To evaluate the potential of the method for clinical use, we studied maternal blood samples from 11 women referred to us for prenatal diagnosis between 15 and 20 weeks of gestation. For simple and effective enrichment of fetal **nucleated** erythrocytes from peripheral maternal **blood**, we combined a triple **density** gradient and magnetic-activated cell sorting (MACS) of anti- CD71 transferrin receptor antibody labeled cells. The isolated cells were analysed by using dual-colour interphase fluorescent in situ hybridization (FISH) with X -, Y -, 18 and 21 - specific DNA probes. Results. Chromosomal abnormalities detected on enriched fetal cells include trisomy 21 and triploidy. Conclusions. Based on the current results it is suggested that the technique described here is a simple, fast, efficient and reliable

6/7/29 (Item 4 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02665755

Utility

METHODS FOR ENRICHING FETAL CELLS FROM MATERNAL BODY FLUIDS

[Blood, centrifuging, gradient solutions adjusted to a specific density, noninvasive prenatal diagnosis]

PATENT NO.: 5,646,004
ISSUED: July 08, 1997 (19970708)
INVENTOR(s): Van Vlasselaer, Peter, Sunnyvale, CA (California), US (United States of America)
ASSIGNEE(s): Activated Cell Therapy, Inc, (A U.S. Company or Corporation), Mountain View, CA (California), US (United States of America) [Assignee Code(s): 37594]
EXTRA INFO: Assignment transaction [Reassigned], recorded June 30, 1998 (19980630)

POST-ISSUANCE ASSIGNMENTS

ASSIGNEE(s): DENDREON CORPORATION, A DELAWARE CORPORATION 291 NORTH BERNARDO AVENUE MOUNTAIN VIEW, CALIFORNIA 94043
Assignor(s): ACTIVATED CELL THERAPY, INC. -- signed: 09/04/1997
Recorded: June 30, 1998 (19980630)
Reel/Frame: 009297/0566
Brief: CHANGE OF NAME
Rep.: DEHLINGER & ASSOCIATES PETER J. DEHLINGER P.O. BOX 60850 PALO ALTO, CA 94306

APPL. NO.: 8-299,468
FILED: August 31, 1994 (19940831)
FULL TEXT: 1024 lines

ABSTRACT

The present invention relates to methods of enriching fetal cells from maternal body fluids. In particular, it relates to the use of a cell-trap centrifugation tube containing a gradient solution adjusted to a specific **density** to enrich for fetal **nucleated** red **blood** cells from maternal **blood**. The tube allows the desired cell population to be collected by decantation after centrifugation to minimize cell loss and maximize efficiency. In addition, the method can be further simplified by density-adjusted cell sorting which uses cell type-specific binding agents such as antibodies and lectins linked to carrier particles to impart a different density to undesired cell populations allowing the fetal cells to be separated during centrifugation in a more convenient manner. The rapid fetal cell enrichment method described herein has a wide range of applications, including but not limited to, gender determination and prenatal diagnosis of genetic diseases without the use of invasive procedures.

6/7/31 (Item 6 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02495812

Utility

DENSITY GRADIENT MEDIUM FOR THE SEPARATION OF CELLS

[Cell separation]

PATENT NO.: 5,489,386
ISSUED: February 06, 1996 (19960206)
INVENTOR(s): Saunders, Alexander M., San Carlos, CA (California), US (United States of America)
ASSIGNEE(s): Applied Imaging, (A U.S. Company or Corporation), Santa Clara, CA (California), US (United States of America) [Assignee Code(s): 36234]
EXTRA INFO: Assignment transaction [Reassigned], recorded July 10, 1997 (19970710)

POST-ISSUANCE ASSIGNMENTS

ASSIGNEE(s): APPLIED IMAGING CORPORATION, A DELAWARE CORPORATION BUILDING F 2340A WALSH AVENUE SANTA, CALIFORNIA 95051
Assignor(s): APPLIED IMAGING CORPORATION, A CALIFORNIA CORPORATION -- signed: 10/04/1996
Recorded: July 10, 1997 (19970710)
Reel/Frame: 8595/0527
Brief: ASSIGNMENT OF ASSIGNOR'S INTEREST
Rep.: TOWNSEND AND TOWNSEND AND CREW LLP KAREN B. DOW TWO EMBARCADERO CENTER 8TH FLOOR SAN FRANCISCO, CA 94111-3834

APPL. NO.: 8-189,509
FILED: January 31, 1994 (19940131)
FULL TEXT: 803 lines

ABSTRACT

A density gradient medium for the isolation and enrichment of rare cells, including fetal nucleated erythrocytes from a peripheral blood sample is described. The medium comprises a colloidal density gradient medium dispersed in a meltable gel. In one aspect of the invention, the density gradient medium is hypertonic to facilitate separation of maternal red blood cells from fetal blood cells.

02431919

Utility

METHOD FOR SEPARATING RARE CELLS FROM A POPULATION OF CELLS

PATENT NO.: 5,432,054
ISSUED: July 11, 1995 (19950711)
INVENTOR(s): Saunders, Alexander M., San Carlos, CA (California), US (United States of America)
Zarowitz, Michael A. M., San Carlos, CA (California), US (United States of America)
Baldwin, Patricia J., Sunnyvale, CA (California), US (United States of America)
ASSIGNEE(s): Applied Imaging, (A U.S. Company or Corporation), Santa Clara, CA (California), US (United States of America)
[Assignee Code(s): 36234]
EXTRA INFO: Assignment transaction [Reassigned], recorded July 10, 1997 (19970710)

POST-ISSUANCE ASSIGNMENTS

ASSIGNEE(s): APPLIED IMAGING CORPORATION, A DELAWARE CORPORATION BUILDING F 2340A WALSH AVENUE SANTA, CALIFORNIA 95051
Assignor(s): APPLIED IMAGING CORPORATION, A CALIFORNIA CORPORATION -- signed: 10/04/1996
Recorded: July 10, 1997 (19970710)
Reel/Frame: 8595/0527
Brief: ASSIGNMENT OF ASSIGNOR'S INTEREST
Rep.: TOWNSEND AND TOWNSEND AND CREW LLP KAREN B. DOW TWO EMBARCADERO CENTER 8TH FLOOR SAN FRANCISCO, CA 94111-3834

APPL. NO.: 8-190,327
FILED: January 31, 1994 (19940131)
FULL TEXT: 1165 lines

ABSTRACT

A method for isolating and enriching rare cells, including fetal nucleated erythrocytes from a peripheral blood sample is described. The method includes two centrifugation steps, the first a bulk separation step to enrich erythrocytes from other blood components. The second centrifugation comprises a colloidal density gradient medium dispersed in a hypertonic meltable gel. In one aspect of the invention, maternal erythrocytes may be hemolyzed prior to the second density gradient centrifugation to provide additional enrichment for fetal nucleated erythrocytes.

6/7/40 (Item 4 from file: 144)
DIALOG(R)File 144:Pascal

07581198 PASCAL No.: 87-0418456

Identification of blast cells in peripheral blood through automatic assessment of nuclear density: a new tool for monitoring patients with acute leukaemia

D'ONOFRIO G; MANCINI S; LEONE G; BIZZI B; MANGO G
Univ. cattolica Sacro Cuore, ist. semeiotica medica, Roma 00168, Italy

Journal: British journal of haematology, 1987, 66 (4) 473-477
ISSN: 0007-1048 CODEN: BJHEAL Availability: CNRS-7597
No. of Refs.: 8 ref.
Document Type: P (Serial) ; A (Analytic)
Country of Publication: United Kingdom

US PAT NO: 5,840,502 [IMAGE AVAILABLE] L5: 4 of 32
TITLE: Methods for enriching specific cell-types by density gradient centrifugation

ABSTRACT:
The present invention relates to methods of enriching for desired cell population from cell sources, such as body fluids, dispersed tissue specimens and cultured cells. In particular, the present invention relates to the use of a cell-trap centrifugation tube containing a specific density gradient solution adjusted to the specific density of a desired cell population to enrich for the desired cell from a cell source. The tube allows the desired cell population to be collected by decantation after centrifugation to minimize cell loss and maximize efficiency. In addition, the method can be further simplified by density-adjusted cell sorting which uses cell type-specific binding agents such as antibodies and lectins linked to carrier particles to impart a different density to the undesired populations in a more convenient manner. The rapid cell enrichment method described herein has a

US PAT NO: 5,766,843 [IMAGE AVAILABLE] L5: 10 of 32
TITLE: Enriching and identifying fetal cells in maternal blood for in situ hybridization on a solid surface

ABSTRACT:
Fetal cells may be obtained from amniocentesis, chorionic villus sampling, percutaneous umbilical cord sampling or in vitro fertilization embryos or products of conception, but are preferably from maternal peripheral **blood**. Fetal cells may be enriched by **density** gradient **centrifugation**. Fetal cells may also be enriched by removing maternal cells with an antibody to a cell surface antigen, e.g. anti-CD45, either immobilized or by fluorescence-activated cell sorting. Fetal cells are also distinguishable from maternal cells by staining, e.g. with a labeled antibody to cytokeratin or to fetal hemoglobin, or for fetal hemoglobin by hematoxylin/eosin, or by in situ hybridization to detect one or more fetal mRNAs, e.g., of fetal hemoglobin or fetoprotein. Amplification may be used in conjunction with the in situ hybridization. Fetal cells circulating in maternal **blood** may be separated by flow cytometry, sorting on their intrinsic light scattering properties. Fetal **nucleated** erythrocytes may be identified by a label for fetal hemoglobin. Fetal cells may be treated to determine genetic characteristics or abnormalities, infectious agents or other properties by nucleic acid hybridization. Genetic abnormalities may include deletions, additions, amplifications, translocations or rearrangements. Multiple abnormalities may also be detected simultaneously, and they may be visually distinguished by color. Kits are provided for the disclosed p

US PAT NO: 5,432,054 [IMAGE AVAILABLE] L5: 27 of 32
TITLE: Method for separating rare cells from a population of cells

ABSTRACT:
A method for isolating and enriching rare cells, including fetal **nucleated** erythrocytes from a peripheral **blood** sample is described. The method includes two **centrifugation** steps, the first a bulk separation step to enrich erythrocytes from other **blood** components. The second **centrifugation** comprises a colloidal **density** gradient medium dispersed in a hypertonic meltable gel. In one aspect of the invention, maternal erythrocytes may be hemolyzed prior to the second **density** gradient **centrifugation** to provide additional enrichment for fetal **nucleated** erythrocytes.

US PAT NO: 5,275,933 [IMAGE AVAILABLE] L5: 28 of 32
TITLE: Triple gradient process for recovering nucleated fetal cells from maternal blood

ABSTRACT:
Nucleated fetal cells such as **nucleated** fetal red **blood** cells are separated from maternal **blood** with a discontinuous triple gradient gel and **centrifugation**. **Nucleated** fetal red **blood** cells are collected at an interface between a gel layer having a **density** in the range of from 1.105 to 1.110 g/mL and the second layer having a **density** in the range of from 1.075 to 1.085 g/mL, and maternal granulocytes are collected at an interface between a gel layer having a **density** in the range of from 1.115 to 1.125 g/mL and a layer having a **density** in the range of from 1.105 to 1.110 g/mL. This allows separation of fetal cells for testing from the maternal **blood** rather than the placenta or amniotic fluid, reducing the risk of sample collection and facilitating routine testing of fetal cells for evidence o

02878095
Utility

METHODS FOR ENRICHING SPECIFIC CELL-TYPES BY DENSITY GRADIENT CENTRIFUGATION

PATENT NO.: 5,840,502
ISSUED: November 24, 1998 (19981124)
INVENTOR(s): Van Vlasselaer, Peter, Sunnyvale, CA (California), US (United States of America)
ASSIGNEE(s): Activated Cell Therapy, Inc , (A U.S. Company or Corporation), Mountain View, CA (California), US (United States of America) [Assignee Code(s): 37594]
APPL. NO.: 8-299,467
FILED: August 31, 1994 (19940831)
FULL TEXT: 1835 lines

ABSTRACT

The present invention relates to methods of enriching for desired cell population from cell sources, such as body fluids, dispersed tissue specimens and cultured cells. In particular, the present invention relates to the use of a cell-trap centrifugation tube containing a specific density gradient solution adjusted to the specific density of a desired cell population to enrich for the desired cell from a cell source. The tube allows the desired cell population to be collected by decantation after centrifugation to minimize cell loss and maximize efficiency. In addition, the method can be further simplified by density-adjusted cell sorting which uses cell type-specific binding agents such as antibodies and lectins linked to carrier particles to impart a different density to the undesired populations in a more convenient manner. The rapid cell enrichment method described herein has a wide range of diagnostic and therapeutic applications.

6/7/28 (Item 3 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02685011

Utility
SEPARATION APPARATUS AND METHOD
[Centrifuging device with closure, constriction and channels for cell separation]

PATENT NO.: 5,663,051
ISSUED: September 02, 1997 (19970902)
INVENTOR(s): Vlasselaer, Peter Van, Sunnyvale, CA (California), US (United States of America)
ASSIGNEE(s): Activated Cell Therapy, Inc , (A U.S. Company or Corporation), Mountain View, CA (California), US (United States of America) [Assignee Code(s): 37594]
EXTRA INFO: Assignment transaction [Reassigned], recorded June 30, 1998 (19980630)

POST-ISSUANCE ASSIGNMENTS

ASSIGNEE(s): DENDREON CORPORATION, A DELAWARE CORPORATION 291 NORTH BERNARDO AVENUE MOUNTAIN VIEW, CALIFORNIA 94043
Assignor(s): ACTIVATED CELL THERAPY, INC. -- signed: 09/04/1997
Recorded: June 30, 1998 (19980630)
Reel/Frame: 009297/0566
Brief: CHANGE OF NAME
Rep.: DEHLINGER & ASSOCIATES PETER J. DEHLINGER P.O. BOX 60850 PALO ALTO, CA 94306

APPL. NO.: 8-570,397
FILED: December 11, 1995 (19951211)

This is a continuation-in-part of U.S. patent application Ser. No. 08-299,469 now U.S. Pat. No. 5,474,687, and a continuation-in-part of application Ser. No. 08-299,465, U.S. Ser. No. 08-299,467 and U.S. Ser. No. 08-299,468, all filed Aug. 31, 1994.

FULL TEXT: 2867 lines

ABSTRACT

Disclosed is an apparatus designed to be used for enriching specific cell types from cell mixtures. The apparatus includes a centrifugable device that includes a constriction defining a lower region and a defined cell separation medium. The constriction prevents mixing between the upper and lower portions of the device. Also disclosed are methods that use precisely defined cell separation media to isolate specific cells from cell mixtures, including CD34 sup + hematopoietic progenitor cells from blood or bone marrow, nucleated fetal cells from maternal blood, specific tumor cells, dendritic cells, natural killer cells, and natural suppressor cells from various body fluids, and for enrichment or depletion of T cell lymphocytes. Also disclosed is a density adjusted cell separation technique used to augment the above apparatus and enrichment methods. The apparatus and enrichment methods are useful in various diagnostic and therapeutic

13/7/9 (Item 7 from file: 654)
DIALOG(R)File 654:US Pat.Full.

02459620

Utility

FLUID COLLECTION, SEPARATION AND DISPENSING TUBE

[Collecting and separating fluid having lighter and heavier **specific gravity** phases, dispensing lighter phase from **tube** having internal **float** of intermediate **specific gravity** which acts as check valve after centrifuging to retain heavy phase]

PATENT NO.: 5,456,885
ISSUED: October 10, 1995 (19951010)
INVENTOR(s): Coleman, Charles M., 958 Washington Rd., Pittsburgh, PA (Pennsylvania), US (United States of America), 15228
Kendrick, William, 34 Willowbrook Dr., Doylestown, PA (Pennsylvania), US (United States of America), 18901
[Assignee Code(s): 68000]
APPL. NO.: 8-90,329
FILED: July 12, 1993 (19930712)
FULL TEXT: 387 lines

ABSTRACT

A tube for collection, separation and dispensation of a two-phase fluid is provided with a elongated rigid tubular container having a fluid entry end a closable end and a float. The float retained within the tubular container is positioned between the light phase and the heavy phase of the fluid on centrifugation thereby separating the two phases. In dispensing the light phase, the float moves to and engages the fluid entry end thereby preventing the heavy phase from escaping out of the tube.

13/7/11 (Item 9 from file: 654)
DIALOG(R)File 654:US Pat.Full.

01900615

Utility

CORRECTION OF BLOOD COUNT TUBE READINGS

PATENT NO.: 4,952,054
ISSUED: August 28, 1990 (19900828)
INVENTOR(s): Levine, Robert A., 31 Pilgrim La., Guilford, CT (Connecticut), US (United States of America), 06437
Wardlaw, Stephen C., 191 N. Cove Rd., Old Saybrook, CT (Connecticut), US (United States of America), 06475
[Assignee Code(s): 68000]
APPL. NO.: 7-303,120
FILED: January 30, 1989 (19890130)
FULL TEXT: 247 lines

ABSTRACT

The method of correcting readings of cell counts in a centrifuged blood sample contained in a transparent tube which also contains a transparent cylindrical float. Light absorption measurements are made at different locations in the tube. A beam of light is directed through the tube and the float and a light absorption reading is taken. A light beam is also directed through the sample above the float and another absorption reading is taken. The ratio of the two readings is determined and compared to a target ratio that is precalculated from an ideal tube and float. Any deviations from the target ratio are proportional to correction factors which are applied to the cell readings taken. A colorant is added to the sample to impart the light absorption capability thereto.

US PAT NO: 5,834,217 [IMAGE AVAILABLE] L2: 1 of 1

DETDESC:

DETD(6)

It . . . energy emissions, and can even be quantified as set forth hereinafter, without interference from any other bands B. When a **blood** sample is assayed, the nature of the red **blood** cells, ie, the fact that they pack when centrifuged in a manner which excludes significant amounts of the plasma, ensures. . . in the plasma layer, and will not interfere with the procedure. When the sample is being analyzed for a particular **nucleated** cell analyte (ie, a cell having a **nucleus**), the sample may have added thereto a non-specific nuclear colorant, such as a DNA-specific stain, as for example HOECHST-3542; 7. . . the sample is centrifuged, the non-analyte nuceated cell bands will form above the red cell layer, and the captured analyte **nucleated** cells will, by virtue of the density of their capture particle, either settle down into the red cell layer or. . . of fluorescence in the sample, one being the white cell or buffy coat layer, and the other being the captured **nucleated** cell analyte located within the red cell layer or above the white cell layer. The captured analyte cell layer can. . . white cell is a particular type of lymphocyte. The lymphocyte-monocyte cell layer in the buffy coat is measured using the **QBC**.RTM. technique, which technique is described in U.S. Pat. No. 4,027,660. The instrument then measures the intensity of the fluorescence emanating. . .

ABSTRACT:
The present invention concerns DNA sequences which are suitable for immortalizing human or animal cells, processes for isolating such DNA sequences as well as processes for producing immortalized human or animal cells using such a DNA.

US PAT NO: 5,663,051 [IMAGE AVAILABLE] L5: 15 of 32
TITLE: Separation apparatus and method

ABSTRACT:
Disclosed is an apparatus designed to be used for enriching specific cell types from cell mixtures. The apparatus includes a **centrifugable** device that includes a constriction defining a lower region and a defined cell separation medium. The constriction prevents mixing between the upper and lower portions of the device. Also disclosed are methods that use precisely defined cell separation media to isolate specific cells from cell mixtures, including CD34.sup.+ hematopoietic progenitor cells from **blood** or bone marrow, **nucleated** fetal cells from maternal **blood**, specific tumor cells, dendritic cells, natural killer cells, and natural suppressor cells from various body fluids, and for enrichment or depletion of T cell lymphocytes. Also disclosed is a **density** adjusted cell separation technique used to augment the above apparatus and enrichment methods. The apparatus and enrichment methods are useful in various diagnostic and therapeutic regimens.

US PAT NO: 5,662,813 [IMAGE AVAILABLE] L5: 16 of 32
TITLE: Method for separation of nucleated fetal erythrocytes from maternal blood samples

ABSTRACT:
A charge-flow separation apparatus (CFS) and method for enriching rare cell populations, particularly fetal cells, from a whole blood sample by separating the rare cell fractions from whole according to the relative charge density and/or the relative binding affinity for a leukocyte depletion solid phase matrix. The enrichment method may be operated stand alone, or as a pre- or post-processing step in conjunction with a charge-flow separation method.

US PAT NO: 5,646,004 [IMAGE AVAILABLE] L5: 17 of 32
TITLE: Methods for enriching fetal cells from maternal body fluids

ABSTRACT:
The present invention relates to methods of enriching fetal cells from maternal body fluids. In particular, it relates to the use of a cell-trap **centrifugation** tube containing a gradient solution adjusted to a specific **density** to enrich for fetal **nucleated** red **blood** cells from maternal **blood**. The tube allows the desired cell population to be collected by decantation after **centrifugation** to minimize cell loss and maximize efficiency. In addition, the method can be further simplified by **density**-adjusted cell sorting which uses cell type-specific binding agents such as antibodies and lectins linked to carrier particles to impart a different **density** to undesired cell populations allowing the fetal cells to be separated during **centrifugation** in a more convenient manner. The rapid fetal cell enrichment method described herein has a wide range of applications, including but not limited to, gender determination and prenatal diagnosis o

US PAT NO: 5,086,784 [IMAGE AVAILABLE] L8: 9 of 22
TITLE: Centrifuged material layer measurements taken in an evacuated tube

ABSTRACT:
Centrifuged material layer measurements are made in an evacuated glass or clear plastic tube which contains a float. When possibly contaminated materials, such as blood, are being tested the use of the evacuated tube allows the measurements to be made without the technician being exposed to the blood. The tubes are large enough to hold approximately one ml of blood, and are filled with an inert gas at low pressure. Dimensional tolerances relative to those of a capillary tube are relaxed for the tube and float due to the larger sample capacity. The cell bands are stabilized by a layer of a flowable material which settles onto the p

US PAT NO: 4,952,054 [IMAGE AVAILABLE] L8: 12 of 22
TITLE: Correction of blood count tube readings

ABSTRACT:
The method of correcting readings of cell counts in a centrifuged blood sample contained in a transparent tube which also contains a transparent cylindrical float. Light absorption measurements are made at different locations in the tube. A beam of light is directed through the tube and the float and a light absorption reading is taken. A light beam is also directed through the sample above the float and another absorption reading is taken. The ratio of the two readings is determined and compared to a target ratio that is precalculated from an ideal tube and float. Any deviations from the target ratio are proportional to correction factors which are applied to the cell readings taken. A colorant is added to the sample to impart the light absorption capability t

US PAT NO: 4,190,328 [IMAGE AVAILABLE] L8: 17 of 22
TITLE: Process for detection of blood-borne parasites

ABSTRACT:
A process for detecting parasites which are found in the blood of a host. A sample of blood is drawn into a **capillary tube** which contains a generally cylindrical mass having a **specific gravity** such that it will **float** in one of the cell layers when the sample is separated by centrifugation. The **specific gravity** of the mass is selected so as to cause the cylindrical mass to combine with the bore wall of the **capillary tube** to form a thin **annular** space in the **capillary tube** into which the parasites will be crowded, thus increasing the concentration of parasites in a restricted area of the centrifuged blood sample and rendering the parasites highly visible. A stain may be used to differentially color the parasites. One specific parasite which can be detected in this manner quickly and inexpensively is heartworm.

US PAT NO: 4,091,659 [IMAGE AVAILABLE] L8: 18 of 22
TITLE: Apparatus for measuring white cell count

ABSTRACT:
Apparatus for measuring the volume of the buffy coat and constituent cell sub-layers in blood. The apparatus includes a **capillary tube** with an insert disposed in the **tube** bore. The insert is formed of a material having a **specific gravity** which will enable it to **float** on the red cell layer of a centrifuged blood sample. The insert is an elongated body having a circular side wall, such as a cylinder, with a tapered upper end. The insert forms an **annular** free space with the **tube** bore into which the white cell layer settles. A small, axial channel is formed in or on the body to allow ready passage of the fluid constituent of the blood sample during centrifugation so as to minimize disruption of the cell layer interfaces which may otherwise occur during c